

Application No.: 08/914,332  
Amendment Dated: August 22, 2003  
Reply to Office Action Dated: April 22, 2003

## **AMENDMENTS TO THE CLAIMS**

Please amend the claims as follows:

### **LISTING OF CLAIMS:**

Claim 1. (Currently amended) A method of producing a biotin vitamer by:

- (a) culturing a bacterium comprising a *Bacillus subtilis* lysine-utilizing diaminopelargonic acid (DAPA) aminotransferase, said culturing taking place in an environment wherein lysine, a lysine analog, or a lysine precursor is exogenously added to the culture to provide a concentration of at least 10 mmoles lysine, lysine analog, or lysine precursor per liter of culture during the entire culturing step; and
- (b) recovering said biotin vitamer.

Claim 2. (Currently amended) A method of producing a biotin vitamer by:

- (a) culturing a bacterium comprising a *Bacillus subtilis* lysine-utilizing DAPA aminotransferase, wherein the lysine biosynthetic pathway is deregulated in said bacterium; and
- (b) recovering said biotin vitamer.

Claim 3. (Currently amended) The method of claim 1 in which the bacterium is engineered to overproduce a *Bacillus subtilis* lysine-utilizing DAPA aminotransferase.

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Claim 4. (Currently amended) The method of claim 2 in which the bacterium is engineered to overproduce a Bacillus subtilis lysine-utilizing DAPA aminotransferase.

Claim 5. (Original) The method of claim 2 or claim 4, wherein lysine, a lysine analog, or a lysine precursor is exogenously added to the culture.

Claim 6. (Previously amended) The method of claim 2 or claim 4, in which lysine, a lysine analog, or a lysine precursor is exogenously added to the culture to provide a concentration of at least 10 mmoles lysine, lysine analog, or lysine precursor per liter of culture during the entire culturing step.

Claim 7. (Original) The method of claim 1, claim 2, claims 3, or claim 4, in which the biotin vitamer is biotin, dethiobiotin, or diaminopelargonic acid (DAPA).

Claim 8. (Original) The method of claim 1, claim 2, claim 3, or claim 4, in which the biotin vitamer is dethiobiotin, and, after recovering the dethiobiotin, the method further comprises converting the recovered dethiobiotin to biotin by a separate fermentation, biochemical reaction, or chemical reaction, and recovering biotin.

Claim 9. (Original) The method of claim 1, claim 2, claim 3, or claim 4, in which the bacterium is resistant to a lysine analog.

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Claim 10. (Original) The method of claim 9, wherein said analog is S-2-aminoethyl-L-cysteine (AEC).

Claim 11. (Currently amended) The method of claim 1 or claim 2, wherein at least one biotin synthetic pathway step, in addition to expression of a polynucleotide encoding a DAPA aminotransferase, the bioA gene is deregulated in said bacterium.

Claim 12. (Original) The method of claim 1, claim 2, claim 3, or claim 4, in which the biotin vitamer is biotin, and the method comprises recovering and purifying the biotin.

Claim 13. (Previously amended) The method of claim 1, claim 2, claim 3, or claim 4, wherein said bacterium is further engineered to produce a S-adenosylmethionine (SAM)-utilizing DAPA aminotransferase.

Claim 14. (Original) The method of claim 13 in which methionine, S-adenosylmethionine (SAM), or an analog of SAM is added to the culture.

Claim 15. (Original) The method of claim 13 wherein lysine, a lysine analog, or a lysine precursor is added to the culture.

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Claim 16. (Original) The method of claim 14, wherein lysine, a lysine analog, or a lysine precursor is added to the culture.

Claim 17. (Previously amended) The method of claim 15 in which lysine or a lysine analog exogenously added to the culture provides a concentration of at least 10 mmoles lysine or lysine analog per liter of culture during the entire culturing step.

Claim 18. (Previously amended) The method of claim 16 in which lysine or a lysine analog exogenously added to the culture provides a concentration of at least 10 mmoles lysine or lysine analog per liter of culture during the entire culturing step.

Claim 19. (Original) The method of claim 13 in which the biotin vitamer is biotin, dethiobiotin, or diaminopelargonic acid (DAPA).

Claim 20. (Original) The method of claim 13 in which the biotin vitamer is dethiobiotin, and, after recovering the dethiobiotin, the method further comprises converting the recovered dethiobiotin to biotin by a separate fermentation, biochemical reaction, or chemical reaction, and recovering biotin.

Claim 21. (Currently amended) The method of claim 13 wherein at least one biotin synthetic pathway step, other than expression of a polynucleotide encoding a DAPA aminotransferase, the bioA gene is deregulated in said bacterium.

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Claim 22. (Original) The method of claim 13 in which the biotin vitamer is biotin, and the method comprises recovering and purifying the biotin.

Claim 23. (Withdrawn) A bacterium engineered to overproduce a lysine-utilizing DAPA aminotransferase and a SAM-utilizing DAPA aminotransferase.

Claim 24. (Withdrawn) The bacterial strains BI90 (ATCC \_\_\_\_) and BI96 (ATCC \_\_\_\_).

Claim 25. (Withdrawn) The bacterium of claim 23, wherein the strain is further engineered to overproduce the biotin vitamer by engineered deregulation of at least one biotin synthetic step, in addition to *bioA* expression.

Claim 26. (Withdrawn) The bacterial strain BI603 (ATCC \_\_\_\_).

Claim 27. (Withdrawn) A bacterium engineered to overproduce a lysine-utilizing DAPA aminotransferase, wherein the bacterium is further engineered to overproduce lysine.

Claim 28. (Withdrawn) The bacterial strain BI641 (ATCC \_\_\_\_) or BI642 (ATCC \_\_\_\_).

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Claim 29. (Withdrawn) A biotin vitamer manufactured by the method of claim 1, claim 2, claim 3, or claim 4.

Claim 30. (Withdrawn) A biotin vitamer manufactured by the method by claim 13.

Claim 31. (Withdrawn) A biotin vitamer manufactured by the method of claim 14.